

Amendments to the Specification:

Please amend the specification as follows:

Please replace paragraph [0004] with the following paragraph:

[0004] Embodiments of the present invention include a peptide-amphiphile composition or its salts comprising a first peptide-amphiphile with a hydrophilic region and an ionic charge, the hydrophilic region having a first biological signal associated with it; a second peptide-amphiphile or addition salt with a hydrophilic region, the hydrophilic region of the second peptide amphiphile having a second biological signal and opposite ionic charge associated with it. The first and second peptides in these peptide-amphiphile composition have oppositely signed charges. The oppositely charged peptide amphiphiles may have the same or magnitude charge. In these compositions the first peptide and second peptide amphiphile or are mixed/combined in a charge equivalent ratio. Preferably the first peptide or second peptide includes a peptide sequence which promotes adhesion of nerve cells and or those that promote axon outgrowth in cells. For example, the first or second peptide amphiphile may include the amino acid sequences YIGSR (SEQ ID NO:3) or IKVAV (SEQ ID NO:4). To promote bonding of self assembled peptide amphiphiles, the first or second peptide amphiphile may include an amino acid with a functional moiety capable of intermolecular covalent bond formation.

Please replace paragraph [0005] with the following paragraph:

[0005] Another embodiment of the present invention includes compositions comprising self-assembled positively-charged peptide-amphiphiles incorporating a first biological signal and negatively-charged peptide-amphiphiles incorporating a second biological signal. The peptide amphiphiles or their salts in these compositions may include amino acids sequence promoting cell adhesion such as IKVAV (SEQ ID NO:3) and YIGSR (SEQ ID NO:4).

Please replace paragraph [0014] with the following paragraph:

[0014] FIG. 1 illustrates the chemical structure of examples of peptide-amphiphiles having opposite charges and unique biological signal portions (SEQ ID NOS 1-2, respectively, in order of appearance);

Please replace paragraph [0018] with the following paragraph:

[0018] The present invention is directed to various modes of self-assembly and controlled self-assembly of charged peptide-amphiphiles. More particularly, preferred embodiments of the present invention are directed to a mixture of two or more charged peptide-amphiphiles which self assemble to form a nanofiber network near physiological conditions. Peptide-amphiphile compositions may include a first peptide-amphiphile having a first biological signal associated therewith and a second peptide-amphiphile having a second biological signal associated herewith. The first and second peptide are oppositely charged; one has a positive ionic charge and the other has a negative ionic charge. The peptide-amphiphile compositions may include amino acids in the peptide

sequence which promotes cell-substrate adhesions, a first biological signal, among nerve cells like YIGSR (SEQ ID NO:3). The peptide-amphiphile composition may include another peptide sequence, a second biological signal, which promotes axon outgrowth in cells like IKVAV (SEQ ID NO:4). The peptide amphiphiles having the unique biological signal may self assemble to form nanofiber network comprised of a positively-charged peptide-amphiphile incorporating the first biological signal and a negatively-charged peptide-amphiphile incorporating the second biological signal.

Please replace paragraph [0023] with the following:

[0023] Table 1 below illustrates representative, non-limiting examples of peptide-amphiphiles with opposite charge and distinct biological signals.

TABLE 1:

<u>SEQ_ID</u>	<u>N-terminus</u>	<u>Peptide (N to C)</u>	<u>C-terminus</u>	<u>Net Charge at pH 7</u>
<u>NO.</u> [[#]]				
1	C16	AAAAGGGEIKVAV	COOH	-1
2	C16	AAAAGGGKYIGSR	NH ₂	+2

Please replace paragraph [0025] with the following paragraph:

[0025] The alkyl tail has been patterned in large part after the original PA described by Hartgerink, *et al*, Science, vol 294, pp 1684, (2001) and PNAS vol 99, pp 5133, (2002), the contents of which are incorporated herein by reference in their entirety, where the carbon chain serves as the hydrophobic component of the amphiphile and creates the slender portion of the molecules' conical shape. The structural peptide sequences described herein provide a number of different functions and consist of various

amino-acid segments each coupled to the hydrophobic tail. The structural segment in an alternative embodiment includes one or more cysteine amino acids which provides assembled fibers with reversible cross-linking potential. Once assembled into nanofibers, the S-H ligands of the cysteines are believed to be arranged near enough one-another that oxidation of the molecule will enable the formation of stable disulfide bonds. While this cross-link provides structural stability for the molecule, it may be reversed with a reducing agent, such as dithiolthreitol (DTT). The alanine-based structure is not cross-linkable, but avoids the problems of premature molecular crosslinking, which may form between unassembled PA molecules in the presence of oxygen (air). This cysteine-free system may be more appropriate for *in situ* biological applications where the environment may be more difficult to regulate. The SLSL (SEQ ID NO:5) modification to the system is expected to lead to a slower assembly of the nanofibers. It is believed that the bulky leucine side chains may require more time to pack into the fiber. A slowed self-assembly may also have greater applications in a functional, *in situ* environment such as an operating room, where it may be advantageous to have delayed formation of the nanofibers. The functional hydrophobic head of the peptide is a relatively bulky, charged segment of the molecule, and it serves as the widest region of the conical molecular geometry. Self-assembly of PA mixtures may also allow for the presentation of different amino acid sequences along the length of an assembled fiber.

Please replace paragraph [0027] with the following paragraph:

[0027] FIG. 1 illustrates the chemical structures of Molecule 1 and Molecule 2 in accordance with a preferred embodiment of the present invention. FIG. 1 also illustrates

the chemical connectivity of a peptide-amphiphile has been described previously indicating three important segments for consideration in the design of the molecule: Segment 1 is generally a simple hydrophobic tail such as an alkyl tail that can be a variety of sizes but should be greater than 6 carbon atoms in length; Segment 2 is a structural segment that includes amino acids that link the alkyl tail to the hydrophilic head group. If cross-linking of peptide amphiphiles or their salts in nanofibers is desired, cysteine amino acids may be utilized in this segment. If cross-linking is not desired, other amino acids such as alanine may be used in this region (e.g. SLSL (SEQ ID NO:5) or AAA as described in more detail herein). The structural segment may also include a flexible linker composed of glycine or other flexible amino acids. In accordance with the present invention, Segment 3 includes the hydrophilic head group and may be comprised of essentially any charged or hydrophilic amino acid such as lysine, arginine, serine, phosphorylated serine, and aspartic acid resulting in a highly charged peptide-amphiphile. As will be discussed further herein, these charged peptide-amphiphiles may be positively or negatively charged and the amino acid sequence similar to biologically relevant signals like IKVAV (SEQ ID NO:4) and YIGSR (SEQ ID NO:3).

Please replace paragraph [0034] with the following paragraph:

[0034] Molecule 1 shown in Fig. 1 contains a portion of the laminin amino acid sequence IKVAV, (Ile-Lys-Val-Ala-Val) (SEQ ID NO:4), which is part of the 19-mer peptide (PA222-2), which has been extensively shown to promote axon outgrowth in neurons. Molecule 2 contains the amino acid sequence YIGSR (SEQ ID NO:3), which has similarly been shown to promote cell-substrate adhesion among nerve cells and also

Application No. 10/668,672
Response and Amendment to Office Action dated October 9, 2007

to play a role in axon guidance. The two molecules can be dissolved in pH-adjusted water at a concentration of about 2-30mg/ml, and preferably about 10mg/mL. Molecule 1 is completely clear at this concentration; Molecule 2 is translucent. A self-supporting gel forms quickly on mixing the two solutions at neutral pH. Examination of this gel by negative stain TEM reveals cylindrical micelles. Self-assembled peptide amphiphiles of the present invention can include other mixtures of charged peptide amphiphiles.

Please replace paragraph [0043] with the following paragraph:

[0043] This technology can be used for a variety of purposes. This system of self-assembling nanofibers may have a number of different potential applications in the biomedical and tissue engineering industry. The complimentary nature of the biological portions of the PA provide potentially synergistic applications. For example, the inclusion of both YIGSR (SEQ ID NO:3) and IKVAV (SEQ ID NO:4) provide heretofore unexpected synergistic applications for nerve regeneration.

Please replace the Sequence Listing with the accompanying Sequence Listing filed concurrently herewith.